Cloning of Full-length Elastin cDNAs from a Human Skin Fibroblast Recombinant cDNA Library: Further Elucidation of Alternative Splicing Utilizing Exon-specific Oligonucleotides

Michael J. Fazio, M.D., David R. Olsen, Ph.D., Eunkyung A. Kauh, B.A., Clinton T. Baldwin, Ph.D., Zena Indik, Ph.D., Norma Ornstein-Goldstein, Ph.D., Helena Yeh, M.S., Joel Rosenbloom, M.D., Ph.D., and Jouni Uitto, M.D., Ph.D.

Departments of Dermatology H, Biochemistry and Molecular Biology, and Pharmacology, Jefferson Institute of Molecular Medicine, Jefferson Medical College, Thomas Jefferson University; and the Center for Oral Health Research, University of Pennsylvania School of Dental Medicine, Philadelphia, Pennsylvania

A human cDNA library was constructed utilizing RNA isolated from cultured skin fibroblasts. Recombinant clones containing elastin sequences were identified by plaque hybridizations with previously characterized human placental elastin cDNAs. Seven positive recombinant clones with inserts of ~3.2–2.2 kb were isolated. Characterization of the clones by restriction endonuclease analysis and dot-blot hybridizations with exon-specific synthetic oligonucleotides demonstrated considerable variability in the primary nucleotide sequence. Dideoxy nucleotide sequencing confirmed this finding. The variability is most likely a result of alternative splicing of exons from the primary elastin transcripts. The two largest clones contained ~1 kb of 3′ untranslated sequence and ~2.2 kb of translated sequence encoding 730 amino acids. Six amino acids, encoded by exon 12A, have not been previously noted in human elastin cDNAs. In addition, these human skin fibroblast clones contained a 49 bp 5′ untranslated sequence. These results demonstrate that there is considerable variability in the processed nucleotide sequence of the elastin mRNAs. These transcripts may code for isoforms of tropoelastin with different biologic properties. *J Invest Dermatol 91:458–464, 1988*

Several lines of evidence suggest that elastic fibers provide resilience and elasticity to tissues, including human skin. These elastic fibers are composed of two biochemically distinct components. The principal component is elastin, a well-characterized connective tissue protein that is responsible for elastic properties. Surrounding the elastin core is the microfibrillar component, which probably consists of several glycoproteins, thus far poorly characterized. (For reviews on elastic fibers, see Refs 1–5).

The biosynthetic precursor of human elastin, known as tropoelastin, has been sized at ~70 kd and contains ~750 amino acids. The primary amino acid sequence of tropoelastin is encoded by a 3.5 kb mRNA and consists of alternating hydrophobic domains (rich in proline, glycine, and valine) and putative crosslink regions (enriched in alanine and lysine [6–10]. The newly synthesized human tropoelastin contains an amino-terminal signal sequence of 26 amino acids and a highly basic carboxy-terminus that contains two cysteinyl residues [6,8].

Several different cell types, including human skin fibroblasts, have been shown to express the elastin gene in culture [9–12]. It has been suggested that altered regulation of elastin gene expression or aberrations in the primary structure of elastin may be the underlying molecular defect in some cutaneous diseases affecting the elastic fibers [1–5]. Thus, the availability of full-length human skin fibroblast elastin cDNAs would be a useful tool to study elastin pathology encompassing at the molecular level.

In the present study, we have constructed a human skin fibroblast cDNA library and screened it for elastin cDNAs. We have been successful in isolating several cDNAs, which unequivocally correspond to human elastin sequences. Two of the cDNAs are ~3.2 kb in size, and contain the complete coding region of elastin mRNA, together with 5′ and 3′ untranslated sequences. Characterization of these and five other human skin fibroblast elastin cDNAs revealed considerable variability in the primary nucleotide sequence resulting from alternative splicing of the primary elastin transcript. These findings may have significant implications in elastic fiber pathology.

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Reprint requests to: Jouni Uitto, M.D., Ph.D., Department of Dermatology, Jefferson Medical College, 1020 Locust Street, M46 Jefferson Alumni Hall, Philadelphia, PA 19107

Abbreviations:
bp: basepairs
kb: kilobases
kd: kilodaltons

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MATERIALS AND METHODS

Construction and Screening of a Human Skin Fibroblast cDNA Library Total RNA was isolated from cultured human skin fibroblasts (JIMM-69; established from a full-term fetus) using guanidinium isothiocyanate extraction followed by CsCl density gradient centrifugation [13], and used for synthesis of the cDNA library [14]. The purified RNA was primed with oligo-dT, and first strand synthesis was catalyzed by cloned Maloney murine leukemia virus reverse transcriptase (BRL). The resultant RNA/DNA hybrid was subjected to RNase H digestion followed by second strand synthesis catalyzed by DNA polymerase I [15]. The cDNAs were blunt-ended, using T4 polymerase, and ligated into the phage vector λZAP (Stratagene, San Diego, CA) using EcoRI linkers. The cDNAs were packaged using GIGA Pack Extract (Stratagene). The bacteriophage library was plated using an *E. coli* strain XL-1-Blue (Stratagene). The cDNA library had $\sim 2 \times 10^7$ independent clones.

Initial screening of approximately 10^6 independent clones of the unamplified cDNA library was carried out in duplicate with two separate probes: a) cHE2, a 2.5 kb human placental elastin cDNA containing ~1.5 kb of translated sequence (7); and b) a 5′, ~400 bp subclone of cHE2 isolated by EcoRI-BamHI double restriction endonuclease digestion. These probes were radiolabeled by nick translation [16] with $\alpha[^{32}P]$dCTP and used to screen the library by plaque hybridization. The hybridization and washing conditions for the library screening were the same as described previously [7].

Clones positive to both the 2.5 kb cDNA and its 5′ subclone, were subjected to plaque purification, and the isolated recombinants were digested with EcoRI endonuclease. Electrophoresis on 1% agarose gel, with comparison to standard DNA markers (New England Bio Labs, Beverly, MA), was used to estimate the size of the inserts.

Characterization of Elastin cDNAs The newly isolated cDNAs were characterized by restriction endonuclease digestions, followed by separation of the DNA fragments on agarose gel electrophoresis. The appropriate DNA fragments were cloned into the phage vector M13 (mp18 and mp19; Boehringer Mannheim, Indianapolis, IN), and nucleotide sequencing was performed using the dideoxy chain termination method [17]. Sequencing primers included the universal M13 17-mer primer, as well as appropriate oligonucleotides synthesized for extension of the sequencing.

Hybridizations with Exon Specific Probes Oligonucleotide sequences specific for individual exons were selected by computer-assisted analysis of the human elastin gene structure [6,18]. The exon-specific oligonucleotides were synthesized using a modification of the phosphite method of Matteucci and Caruthers [19] employing a MilliGen (Bedford, MA) programmable synthesizer. The synthetic oligonucleotides were purified by reverse phase high-pressure liquid chromatography (Varian 5000).

Exon-specific synthetic oligonucleotides were radioactively labeled at the 5′-end, with $\gamma[^{32}P]$dATP, by a phosphate exchange reaction catalyzed by T4 polynucleotide kinase. For elucidation of the presence or absence of a specific exon sequence within the dermal fibroblast clones, ~100 ng of the insert cDNA was denatured, dotted onto nitrocellulose filters, and hybridized with 10 ng of the radiolabeled exon-specific oligonucleotide probe. Filter prehybridization was performed in a solution consisting of 0.9 M NaCl, 90 mM sodium citrate (pH 7.0), 0.5% sodium dodecylsulfate, 100 μg/ml denatured salmon sperm DNA, 0.1% polyvinyl pyrrolidine, 0.1% bovine serum albumin, and 0.1% Ficoll, at 42°C for 2 h. The hybridization, following addition of the labeled synthetic oligonucleotide probe, was carried out for 16 h in the same solution. The filters were washed at a final stringency of 0.15 M NaCl, 15 mM sodium citrate, at 55°C for 60 min.

The specificity of hybridization with exon-specific oligonucleotides was confirmed by Northern transfer analysis, of poly,(A)$^+$RNA isolated from cultured human skin fibroblasts by oligo-dT cellulose chromatography [6]. In dot-blot hybridizations with an exon 1-specific oligonucleotide, negative controls were provided by an elastin genomic DNA fragment (HEL3) lacking sequences corresponding to exon 1 [6]. Otherwise, positive and negative controls were provided by cDNAs, which have been shown previously to contain or lack the exon sequences to be tested [7].

RESULTS

A human skin fibroblast cDNA library was constructed and screened for elastin cDNAs using human placental elastin cDNA probes previously isolated and characterized [7]. Using both a 2.5-kb cDNA (cHE2) and its 5′-subclone, seven independent clones, positive to both probes, were isolated and characterized. Following plaque purification, EcoRI endonuclease digestion was used to release the insert from the cloning vector. Estimates of the insert size revealed that the two largest clones (cHDE1 and cHDE2) were ~3.2 kb. One clone, cHDE3, was slightly shorter, ~3.1 kb in size, while the remaining 4 clones varied between 2.2 and 2.8 kb in size.

The newly isolated cDNAs were characterized by Northern transfer analysis, restriction endonuclease digestions, dot-blot hy-

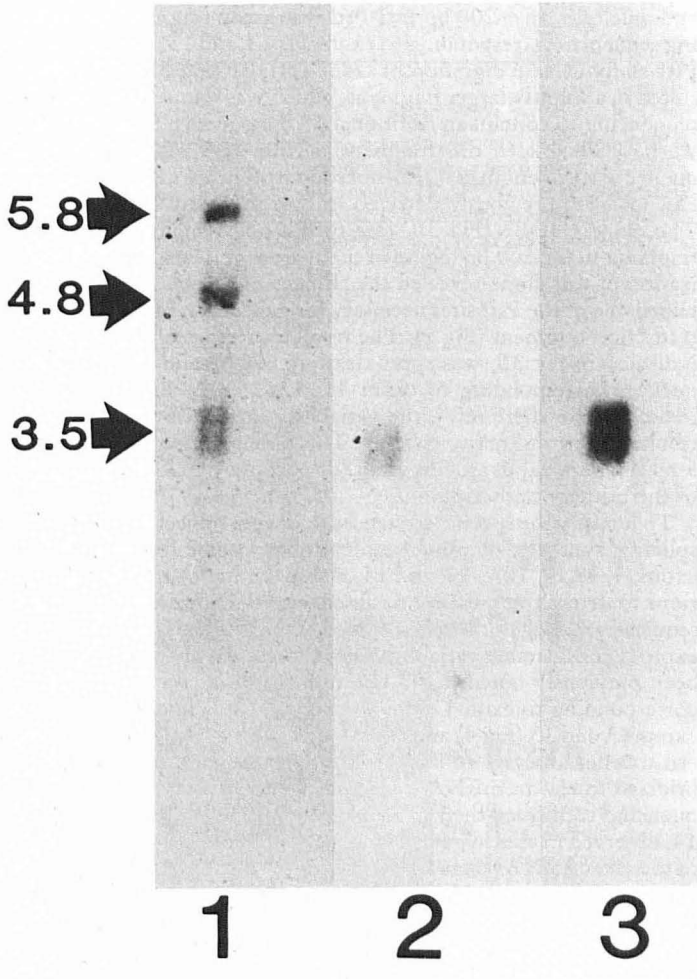

Figure 1. Northern transfer analysis of RNA isolated from cultured human skin fibroblasts. The picture depicts autoradiograms of radioactively labeled elastin cDNA — mRNA hybrids. Each lane contains 1 μg of poly (A)$^+$RNA which has been electrophoresed on a 1% denaturing agarose gel and transferred to nitrocellulose filters. Filters were hybridized with a combination of a proα1 (I) collagen cDNA (Hf677) and the elastin cDNA clone cHDE1 *(lane 1)*; and synthetic oligonucleotides specific for exons 1 and 4A, respectively *(lanes 2 and 3)*. The 5.8 and 4.8-kb bands correspond to the established [35] sizes of the polymorphic proα1 (I) collagen transcripts. The bands in the 3.5-kb region correlate with the known size of the full-length elastin mRNA.

bridizations with exon-specific synthetic oligonucleotides, and dideoxy nucleotide sequencing.

When Northern blots of total RNA or poly(A)$^+$RNA isolated from human skin fibroblasts were hybridized to ^{32}P-labeled dermal fibroblast cDNAs, under conditions previously described [7,9,10], mRNA transcripts in the range of 3.5 kb were revealed (Fig 1*A*). As noted by us [6,7,10] and others [11,12], the band in the 3.5-kb region was relatively broad, suggesting microheterogeneity. As discussed below, we suggest that this variability is a reflection of alternative splicing and leads to synthesis of a population of mRNAs with slightly variable sizes.

The newly isolated cDNAs were characterized by restriction endonuclease digestions (Figs 2 and 3). As indicated previously [6,7], human elastin cDNAs contain a single restriction enzyme site for BamHI and HindIII endonucleases, respectively. Examination of the seven human skin fibroblast elastin cDNAs by double digestion with these enzymes indicated that the resulting BamHI-HindIII fragments varied in size (Fig 3*A*). The difference between the smallest and largest subfragment was approximately 100 nucleotides. Smaller DNA fragments were obtained by PstI restriction endonuclease digestion (Figs 2 and 3*B*), which allowed more detailed delineation of the exact regions of variability. When the clones cHDE4 through 7 (Fig 3*B, lanes C–F*) were digested with PstI endonuclease, an ~200 bp PstI-PstI restriction fragment, containing sequences corresponding to exons 11, 12, and 13, was observed. PstI endonuclease digestion of clone cHDE1 (Fig 3*B, lane A*) resulted in a slightly larger fragment, which was found by nucleotide sequencing to contain an additional 18 bp sequence 5′ of exon 12; we have designated this fragment as exon 12A. An identical sequence was present in cHDE2 (not shown). The exon 12A sequence has not been previously detected in human elastin cDNAs [6,7]. The clone cHDE3 (Fig 3*B, lane B*) did not contain a restriction fragment in the 200 bp region of the agarose gel. Further characterization of this clone revealed the absence of exon 13, which contained one of the PstI sites necessary for generation of the ~200 bp restriction fragment (Fig 2). The resultant restriction fragment, as indicated in Fig 3*B,* was approximately 600 bp and contained sequences corresponding to exons 11, 12, 15, 16, 17, and 18. As indicated elsewhere [6,7], the variability in cDNA fragment size resulted from alternative splicing. This phenomenon results in differential removal of specific exons or portions of exons from some of the primary transcripts.

To identify the specific sequences that were subject to alternative splicing, synthetic oligonucleotide probes specific for six different exons, 1, 4A, 4, 10A, 13, and 14, were used in dot-blot hybridizations to detect the presence or absence of the corresponding exon sequences in each of the seven cloned cDNAs (Fig 4) [6]. Except for exon 1, considerable variability in the presence of these exons has been previously noted [6,7]. The results indicated that sequences corresponding to exon 1 were always present, while variability in exons 4A and 13 (Fig 4) and exon 4 (not shown) was noted. Northern transfer analyses confirmed that these probes specifically hybridized to elastin mRNA transcripts (Fig 1*B*), and nucleotide sequencing confirmed their hybridization specificity. Exons 10A and 14, observed in elastin genomic sequences were not detected in any of the seven cDNAs (Figs 2, 4, and Table I). Furthermore, Northern transfer analysis of RNA isolated from human skin fibroblasts with oligonucleotides specific for exons 10A and 14 failed to yield a detectable signal, even after extended autoradiography of the Northern filters (not shown). These observations suggest that exons 10A and 14 of the elastin gene [6] are not expressed in cultured human skin fibroblasts.

The newly isolated cDNAs were sequenced after subcloning into the phage vector M13. The 3′ untranslated region was ~1 kb and revealed a high degree of homology with previously published sequences in the corresponding region [18]. Six of the clones extended 3′ beyond the first of the two polyadenylation signals recognized in the human elastin gene [18]. The composite cDNA, encompassing the entire translated segment of the elastin mRNA, consisted of 2190 nucleotides encoding 730 amino acids, and included a hydrophobic, 26 amino acid, signal sequence (Fig 5) [6]. In the coding segment, hydrophobic domains usually alternated with putative cross-link regions. Each domain was representative of a distinct exon in the elastin gene [6,20–22]. An exception to this repeating pattern was a segment covering amino acid residues 206–244, which was relatively rich in tyrosine.

An interesting, previously unpublished observation was the presence of an 18 bp 5′ extension of exon 12, designated here as exon 12A, in two clones (cHDE1 and cHDE2) (Fig 5). As discussed below, this additional sequence is incorporated into the mRNA by utilization of a different splice junction at the end of exon 12. In addition, two of the clones (cHDE1 and cHDE2) contained a 49 bp 5′ untranslated sequence, not previously noted in human aortic or placental cDNAs (Fig 5).

DISCUSSION

In the present study, we have constructed a human skin fibroblast cDNA library and successfully screened it for human elastin cDNAs. The two largest clones (cHDE1 and cHDE2), ~3.2 kb in size, contained ~1 kb of 3′ untranslated sequences. These two cDNAs apparently utilized the most 5′ of the two polyadenylation signals noted in the elastin gene [18]. The composite dermal fibroblast cDNA accounted for the entire translated portion of the elastin mRNA, encoding 730 amino acids. Because of the relative abundance of glycine, the calculated molecular weight for the encoded tropoelastin is only 63,326 daltons. The encoded amino acid sequence contained several previously well-characterized domains [6,7]: a) Hydrophobic domains rich in proline, glycine, and valine, which are responsible for the elastic properties of elastin; b) cross-link domains rich in lysyl residues, which undergo oxidative deamination to form desmosine and isodesmosine, covalent cross-link compounds characteristic of elastin [20]; c) a highly basic carboxy-terminus containing two cysteinyl residues that may play a role in the assembly of tropoelastin into the elastic fiber network; d) a characteristically hydrophobic signal sequence consisting of 26 amino acids; and e) a tyrosine-rich domain (amino acid residues 206–244). In addition, a previously unidentified sequence, encoding 6 amino acids 5′ of exon 12 (exon 12A), was delineated (Figs 2 and 5). It is noteworthy that the latter sequence contained an asparagine residue, not noted elsewhere along the translated sequence. Although asparagine (N) can be an acceptor for N-glycosylation, the residue encoded by exon 12A is not a candidate for glycosylation because the required motif, N-X-S/T (X = any amino acid, S = serine, T = threonine), was absent [23]. Finally, a 49 bp 5′ untranslated nucleotide sequence was identified and was found to be different from a comparable region of human aortic elastin cDNAs [6]. Variability in this region may be due to alternative splicing of untranslated sequences of the elastin mRNA. Correlation of cDNA with genomic sequences and primer extension sequencing of elastin mRNA are currently being studied to clarify the significance of this finding.

Characterization of the dermal elastin cDNAs revealed considerable variability in the primary nucleotide sequence, which, as discussed below, may result from alternative splicing of the primary elastin transcripts [6,7,22]. Comparison of the alternative splicing pattern with similar information available on human aortic and placental elastin cDNAs showed that the same exons were frequently subject to alternative splicing (Table I) [6,7]. However, a clear difference in the splicing pattern was noted when compared to bovine ligamentum nuchae elastin mRNAs (Table I) [22]. Although the splicing pattern appears to demonstrate minimal tissue specific differences and more significant species specific differences, definitive conclusions based on the limited number of cDNAs studied here would be inappropriate. Preliminary data utilizing S-1 nuclease analysis on bovine ligamentum nuchae elastin mRNAs suggest that alternative splicing patterns may be developmentally regulated [24].

Alternative splicing is a phenomenon that is known to occur in the processing of a large number of diverse genes, including other extracellular matrix genes [26,27], besides elastin. Several lines of

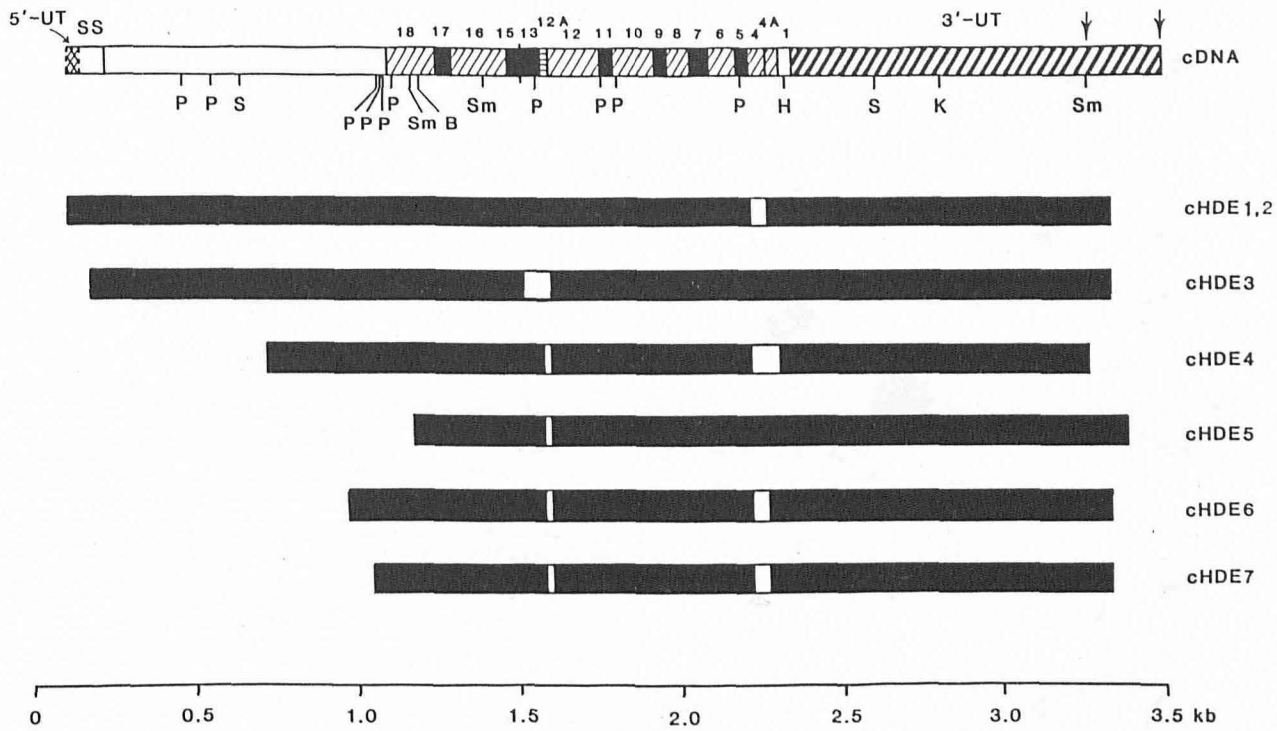

Figure 2. Restriction endonuclease map of the composite dermal fibroblast cDNA. Schematic presentation of the seven clones, cHDE1 through 7, is depicted below the composite cDNA. *Open boxes* represent exons that are differentially spliced from the respective clones. The *bottom line* represents a 3.5-kb scale corresponding to the full-length human elastin mRNA. The composite cDNA encompasses *a)* ~1 kb 3′ untranslated (3′-UT) sequence *(thick lines)* with two polyadenylation signals (↓); *b)* ~2.2-kb translated sequence which includes two cysteinyl residues in exon 1, hydrophobic exons *(thin lines)*, putative cross-link exons *(solid)*, and the 78-bp signal sequence *(SS)*; and *c)* at 49-bp 5′ untranslated (5′-UT) sequence *(hatched)*. Restriction endonucleases used: BamHI *(B)*, HindIII *(H)*, KpnI *(K)*, PstI *(P)*, SacI *(S)*, and SmaI (Sm).

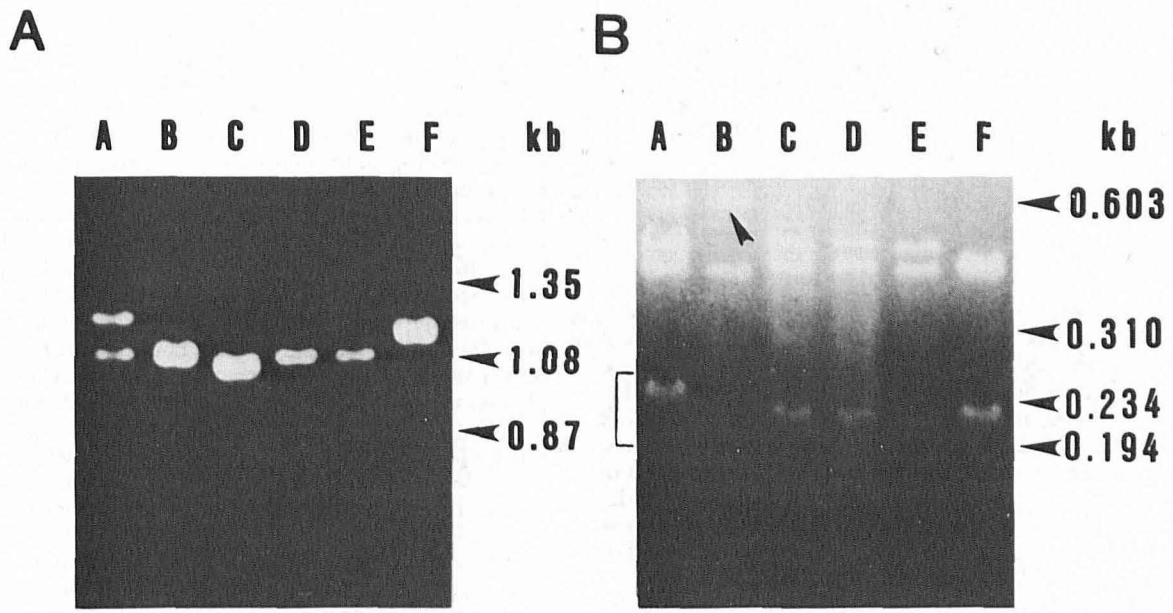

Figure 3. Agarose gel electrophoresis of the dermal elastin cDNAs after restriction endonuclease digestions with *Panel A* (BamHI and HindIII), or *Panel B* (PstI). *Lane A:* cHDE1; *Lanes B-F:* cHDE3 through 7, respectively. Migration positions of standard DNA markers are indicated on the right side of each gel. *Panel A, lane A* contains two separate restriction fragments; the *lower band* is the BamHI-HindIII fragment, while the *upper band* corresponds to the 5′ BamHI-EcoRI fragment of the cDNA. The size of the BamHI-HindIII fragment, which encompasses exons 1-18 (Fig 2), is variable in the different cDNAs; *Panel B:* PstI digest of the same cDNAs as in *Panel A*. The *bracketed region* and *arrowhead (lane B)* illustrate restriction fragment polymorphism of these cDNAs. Note that *Lane B* of *panel B* has a fragment of ~600 bp *(arrowhead)* due to absence of exon 13 in clone cHDE3.

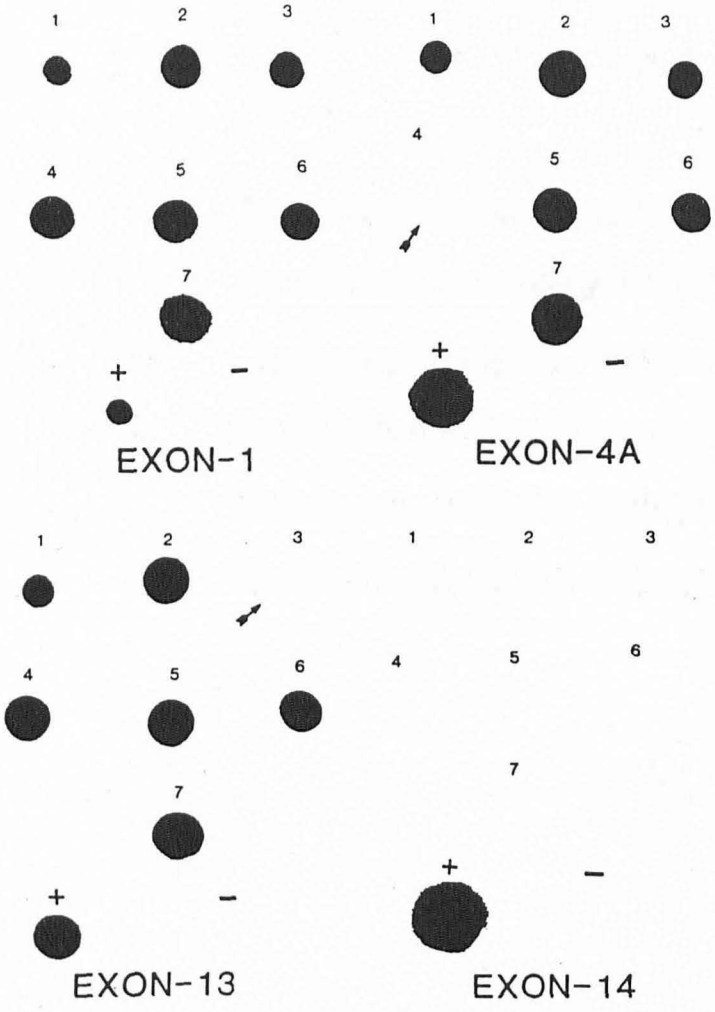

Figure 4. Dot-blot hybridizations of dermal fibroblast cDNAs with exon-specific oligonucleotides. Insert DNA, 100 ng, corresponding to cHDE1 through 7, indicated by the number above each dot, was denatured and applied to nitrocellulose filters. The filters were hybridized with ~10 ng of radiolabeled synthetic oligonucleotides specific for exons 1, 4A, 13, or 14. The autoradiogram indicates that exon 1 was present in all seven human dermal elastin cDNAs; exon 4A was absent from cHDE 4 *(arrow)*; exon 13 was absent from cHDE3 *(arrow)*; exon 14 was not found in any of the dermal fibroblast clones. Positive (+) and negative (−) controls were provided by previously sequenced genomic or cDNA clones [6,7].

Table I. Pattern of Alternative Splicing of Human and Bovine Elastin mRNAs

	Variability of Alternative Splicing[a]			
Exon no.	Human skin[b] fibroblast	Human[b] placental	Human fetal[b] aortic	Bovine fetal ligamentum[c] nuchae
1	100(7)	100(8)	100(11)	100
4A	86(7)	86(7)	100(11)	30–50
4	29(7)	33(6)	45(11)	100
6	100(7)	100(5)	100(11)	98
9	100(7)	100(5)	100(4)	50–95
10A	0(7)	0(5)	9(11)	0
13	86(7)	75(4)	55(9)	94
14	0(7)	0(4)	0(9)	100
Tyr-rich	100(4)	100(1)	100(4)	93–97

[a]Seven independent elastin cDNAs were isolated from human skin fibroblast library in this study and tested for the presence or absence of exon sequences by hybridization with exon-specific oligonucleotide probes, as shown in Fig 4. The alternative splicing pattern was compared with information on human placental [7] and human fetal aortic [6] elastin cDNAs, as well as bovine ligamentum nuchae [24] elastin mRNAs.

[b]Values are expressed as percent of cDNAs tested that contain the exon sequences. Parentheses indicate the number of cDNAs studied.

[c]Values are based on S-1 nuclease mapping of mRNA isolated from ligamentum nuchae. Because the alternative splicing may be developmentally regulated [24], certain exons show a range of values depending on the age of the animals used for isolation of mRNA.

evidence suggest that differences in the primary sequence noted among the human elastin cDNAs indeed result from alternative splicing. First, the copy number of the elastin gene in the haploid human genome is 1 [28,29], and, therefore, all mRNAs reflect the expression of the same gene. Secondly, the nucleotide sequences in all clones studied here were identical, with the exception of the variably omitted regions. Also, a complete homology among human elastin cDNAs isolated from skin fibroblast, placental [7], and fetal aortic [6] cDNA libraries has been noted.

The consequences of alternative splicing of elastin mRNAs are not apparent at this point. However, in fibronectin, the alternative splicing has been shown to lead to synthesis of isoforms of the protein, with different physical, chemical, and functional properties [26]. Thus, it is likely that different isoforms of elastin could be synthesized on the basis of differences in the coding regions of the mRNAs. Because some of the regions affected by alternative splicing contain lysyl residues (exon 13), potentially involved in cross-linking, the covalent intermolecular crosslinks between tropoelastin polypeptides may be affected. Thus, it is conceivable that different isoforms of elastin would confer altered biologic properties to the elastic fiber network. In support of the latter possibility is the observation that different isoforms of elastin have been shown to be synthesized by various cell types [30–33], although it is not definitely proven that these isoforms are incorporated into functional elastic fiber network in vivo.

Another implication of differential splicing concerns the potential for elastin to be altered in diseases [1–5]. Specifically, some of the exons, such as exon 1, have been found to be invariably present in the human elastin cDNAs examined thus far, suggesting that the corresponding sequences of the protein may play a critical role in elastin fibrillogenesis. Furthermore, a deletion or insertion in the elastin gene, to be able to result in a disease, apparently must be relatively large or affect critical sequences or domains. This conclusion is based on the observation that normal variability, resulting from alternative splicing, within the elastin polypeptides can be calculated to be as large as 30 amino acids. In addition to potential structural mutations in the elastin gene, many of the elastin diseases may involve regulatory alterations. For example, regulatory aberrations could lead to excessive accumulation of elastic fibers, as in pseudoxanthoma elasticum [34] and Buschke-Ollendorff syndrome [5], or to deficient elastic fiber formation, as in some forms of cutis laxa [10]. These conditions are currently being studied by using the newly developed cDNAs for hybridizations on the mRNA level.

In summary, we have been successful in isolating full-length elastin cDNAs which correspond to sequences in human skin fibroblast elastin. These cDNAs provide a useful tool to study elastin gene expression in human skin fibroblast cultures and to elucidate its alterations in diseases.

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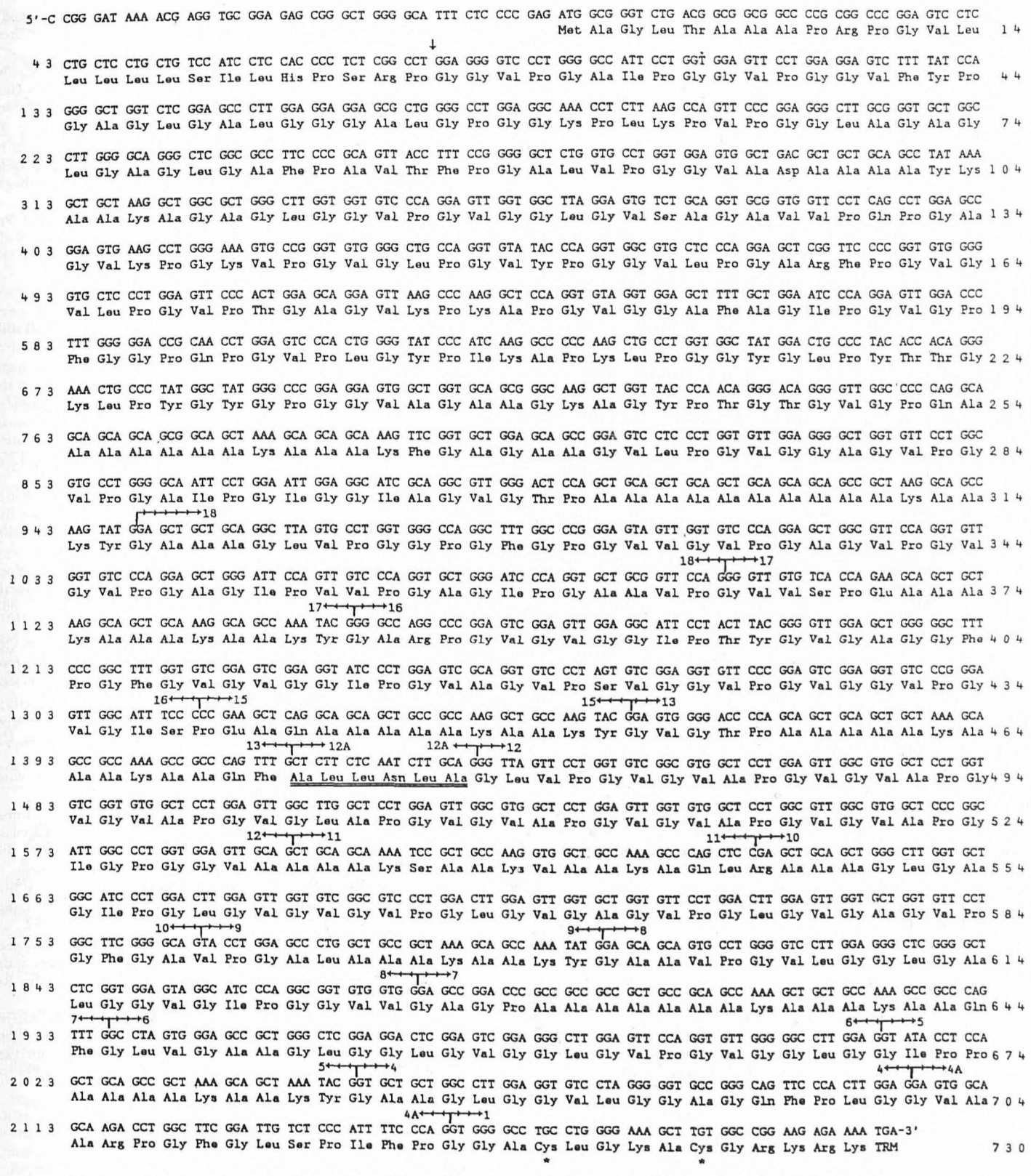

Figure 5. Nucleotide sequence of the composite human dermal fibroblast elastin cDNA. *A:* The translated region (base pairs 1 through 2190) encodes 730 amino acids (shown on the *right side* of the figure). Exon 12A, a previously undetected 18-bp sequence, encoding 6 amino acids, is underlined. *Arrow* indicates the site of cleavage by signal peptidase. The exon boundaries, as deduced from known genomic sequences [6], are indicated. Note the presence of two cysteinyl residues (*asterisk*) in exon 1 and a previously unpublished 49-bp 5′ untranslated sequence. In addition to sequences shown, ~1 kb of 3′ untranslated region was present and found to be identical to previously published sequences [18] TRM, termination codon.

REFERENCES

1. Sandberg LB, Soskel NT, Leslie JG: Elastin structure, biosynthesis, and relation to disease states. New Engl J Med 304:566–579, 1981
2. Davidson JM: Elastin: structure and biology In Uitto J, Perejda A (eds.). Connective Tissue Disease: Molecular Pathology of the Extracellular Matrix. New York, Marcel Dekker, 1987, pp 29–54
3. Rosenbloom J: Elastin: relation of protein and gene structure to disease. Lab Invest 151:603–623, 1984
4. Uitto J: Elastic fibers in cutaneous diseases. Current Concepts Skin Dis 6:19–23, 1985
5. Uitto J, Shamban A: Heritable skin disease with molecular defects in collagen or elastin. Dermatol Clin 5:63–84, 1987
6. Indik Z, Yeh H, Ornstein-Goldstein N, Sheppard P, Anderson N, Rosenbloom JC, Peltonen L, Rosenbloom J: Alternative splicing of human elastin mRNA demonstrated by sequence analysis of cloned genomic and complementary DNA. Proc Natl Acad Sci USA 84:5680–5684, 1987
7. Fazio MJ, Olsen DR, Kuivaniemi H, Chu M-L, Davidson JM, Rosenbloom J, Uitto J: Isolation and characterization of human elastin cDNAs and age-associated variation in elastin gene expression in cultured skin fibroblasts. Lab Invest 58:270–277, 1988
8. Rosenbloom J, Weinbaum G, Abrams W, Ornstein-Goldstein N, Indik Z, Kucich U: Newly determined carboxy terminal sequences in tropolelastin: immunologic identification in insoluble elastin. Collagen Relat Res 6:423–433, 1986
9. Giro GM, Oikarinen AI, Oikarinen H, Sephel G, Uitto J, Davidson JM: Demonstration of elastin gene expression in human skin fibroblast cultures and reduced tropoelastin production by cells from a patient with atrophoderma. J Clin Invest 75:672–678, 1985
10. Olsen DR, Fazio MJ, Shamban AT, Rosenbloom J, Uitto J: Cutis laxa: Reduced elastin gene expression in skin fibroblast cultures as determined by hybridizations with a homologous cDNA and an exon 1 specific oligonucleotide. J Biol Chem 263:6465–6468, 1988
11. Sephel GC, Buckley A, Davidson JM: Developmental initiation of elastin gene expression by human fetal skin fibroblasts. J Invest Dermatol 88:732–735, 1987
12. Sephel GC, Davidson JM: Elastin production in human skin fibroblast cultures and its decline with age. J Invest Dermatol 86:279–285, 1986
13. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294–5299, 1979
14. Huynh TV, Young RA, Davis RW: DNA cloning techniques: a practical approach. Oxford, IRL Press, 1984, pp 49–78
15. Gubler U, Hoffman BJ: A simple and very efficient method for generating cDNA libraries. Gene 25:263–269, 1983
16. Maniatis T, Jeffrey A, Kleid DG: Nucleotide sequence of the rightward operator of phage lambda. Proc Natl Acad Sci USA 72:1184–1188, 1975
17. Sanger F, Nicklen S, Coulson AR: DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 72:5463–5467, 1977
18. Indik Z, Yoon K, Morrow SD, Cicila G, Rosenbloom JC, Rosenbloom J, Ornstein-Goldstein N: Structure of the 3′ region of the human elastin gene: great abundance of ALU repetitive sequences and few coding sequences. Connect Tissue Res 16:197–211, 1987
19. Matteucci MD, Caruthers MH: Synthesis of deoxyoligonucleotides on a polymer support. J Am Chem Soc 103:3185–3191, 1981
20. Raju K, Anwar RA: Primary structures of bovine elastin a, b, and c deduced from the sequences of cDNA clones. J Biol Chem 262:5755–5762, 1987
21. Cicila G, Yoon K, Ornstein-Goldstein N, Indik ZK, Boyd C, May M, Cannizzaro LA, Emanuel BS, Rosenbloom J: Elastin gene structure and function. In Reddi AH (ed.). Extracellular matrix: Structure and function. New York, Alan R. Liss, 1984, pp 333–350
22. Yeh H, Ornstein-Goldstein N, Indik Z, Sheppard P, Anderson N, Rosenbloom JC, Cicila G, Yoon K, Rosenbloom J: Sequence variation of bovine elastin mRNA due to alternative splicing. Collagen Relat Res 7:235–247, 1987
23. Hart GW, Brew K, Grant GA, Bradshaw RA, and Lennarz WJ: Primary structural requirements for the enzymatic formation of the N-glycosidic bond in glycoproteins. J Biol Chem 254:9747–9753, 1979
24. Anderson N, Yeh H, Yoon K, Rosenbloom JC, Rosenbloom J, Ornstein-Goldstein N: Determination of multiple alternative splice sites of bovine elastin mRNA by S-1 mapping (abstr). J Cell Biol 105:213, 1987
25. Breitbart RE, Andreadis A, Nadal-Ginard B: Alternative splicing: a ubiquitous mechanism for the generation of multiple protein isoforms from single genes. Ann Rev Biochem 56:467–495, 1987
26. Kornblihtt RR, Umezawa K, Vibe-Pedersen K, Baralle FE: Primary structure of human fibronectin: differential splicing may generate at least 10 polypeptides from a single gene. EMBO J 4:1755–1759, 1985
27. Pihlajaniemi T, Myllylä R, Seyer J, Kurkinen M, Prockop DJ: Partial characterization of a low molecular weight human collagen that undergoes alternative splicing. Proc Natl Acad Sci USA 84:940–944, 1987
28. Emanuel BS, Cannizzaro L, Ornstein-Goldstein N, Indik ZK, Yoon K, May M, Oliver L, Boyd C, Rosenbloom J: Chromosomal localization of the human elastin gene. Am J Hum Genet 37:873–882, 1985
29. Olliver L, LuValle PA, Davidson JM, Rosenbloom J, Matthew CG, Beater JA, Boyd CD: The gene coding for tropoelastin is represented as a single copy sequence in the haploid sheep genome. Collagen Relat Res 7:77–89, 1987
30. Barrineau LL, Rich CB, Przybyla A, Foster JA: Differential expression of aortic and lung elastin genes during chick embryogenesis. Develop Biol 87:46–51, 1981
31. Rich CB, Foster J: Isolation of tropoelastin a from lathyritic chick aortae. Biochem J 217:581–584, 1984
32. Mecham RP, Morris SL, Levy BD, Wrenn DS: Glucocorticoids stimulate elastin production in differentiated bovine ligament fibroblasts but do not induce elastin synthesis in undifferentiated cells. J Biol Chem 259:12414–12418, 1985
33. Wrenn DS, Parks WC, Whitehouse LA, Crouch EC, Kucich V, Rosenbloom J, Mecham RP: Identification of multiple tropoelastins secreted by bovine cells. J Biol Chem 262:2244–2249, 1987
34. Olsen DR, Lebwohl M, Fazio MJ, Uitto J: Enhanced elastin gene expression in pseudoxanthoma elasticum fibroblast cultures as determined by elastin mRNA steady-state levels (abstr). Clin Res 36:378, 1988
35. Uitto JJ, Perejda AJ, Abergel RP, Chu M-L, Ramirez F: Altered steady-state ratio of type I/III procollagen mRNAs correlates with selectively increased type I procollagen biosynthesis in cultured keloid fibroblasts. Proc Nat Acad Sci USA 82:5935–5939, 1985